DNA Architectures for Templated Material Growth

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14. ABSTRACT

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ABSTRACT

A methodology that allows for the coupling of biology and electronic materials is presented, where double stranded DNA serves as a template for electronic material growth. Self-assembled DNA structures allow for a variety of patterns to be achieved on the nanometer size scale. These DNA architectures allow for feature sizes that are difficult to achieve using conventional patterning techniques. Herein, the procedures for the creation of self-assembled DNA nanostructures in aqueous and non-aqueous media are described, and these structures are subsequently deposited onto substrates of interest. DNA self assembly under non-aqueous conditions has yet to be presented in literature, and is necessary if unwanted oxidation of certain electronic substrates is to be avoided. Solubilization of the DNA in non-aqueous solvents is achieved by replacing charge stabilizing salts with surfactants. Retention of DNA hierarchical structure under both conditions will be presented by observing the structures using AFM imaging and circular dichroism spectroscopic studies.

Keywords: DNA, Directed Assembly, Nanoarchitectures, Nanomaterials, DNA Templated Assembly, AFM

1. INTRODUCTION

Recent advances in science and engineering have led researchers to look towards nature to realize the next level of technological innovation. Specifically, DNA has been a focus of many studies because it is well characterized, relatively inexpensive, easy to synthesize, self-sorting, and readily modified with a variety of functional groups. Additionally, DNA is programmable at the molecular level to pre-organize into well defined secondary and tertiary structures and its size is to scale with traditional nanomaterial building blocks (Figure 1). These characteristics make DNA an ideal target for use as templates for materials applications and for biologically enabled electronics and devices such as: capacitors, sensors, wires, electro-optic modulators, waveguides, semi-conductor materials, radio frequency (RF) shielding, electromagnetic interference (EMI) shielding materials, field effect transistors (FET), energy storage materials, and organic light emitting diodes (OLED).[1] The rapid development in this field has led to a new term being coined to describe it: biotronics.[2]
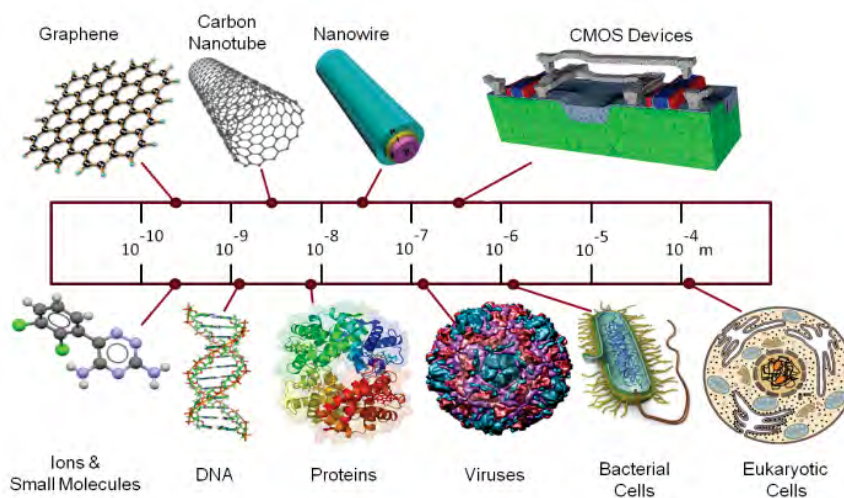

Figure 1. Biological objects and 1D nanomaterials. Diagram compares typical sizes of several biological and nanomaterial building blocks.

This paper will report on a potential application for DNA architectures for use in materials research and discuss potential future applications. These architectures are illustrated to retain their structure when transitioned from aqueous to non-aqueous environments.

1.1   DNA Nanoarchitectures

Structural DNA nanotechnology has seen the development of a rich toolbox of building blocks over the last two decades.[3-8] These tools allow for the creation of a variety of structural architectures with different mechanical properties and allow the assembly of a variety of 2D and 3D structures with exquisite control of periodicity and complexity. (Figure 2) These DNA assemblies range from simple geometric shapes to more complex higher order structures, all varied by use of the genetic code. Although the field has progressed immensely since it was first envisioned by Ned Seeman in the early 1980's, some scientists in the field think the research area lacks widespread utility.[9] In order for more widespread utility of these structures in functional materials like electronic devices a versatile interface that integrates the biological toolkit with micro- and nano-electronic circuitry is needed. Additionally, many electronic device manufacturing is typically done in a non-aqueous environment which precludes the use of biological materials directly as a component of these devices. In this paper we will address one issue of the biological interface by complexing the DNA with a surfactant which allows it to be soluble in a variety of non-aqueous solvents.
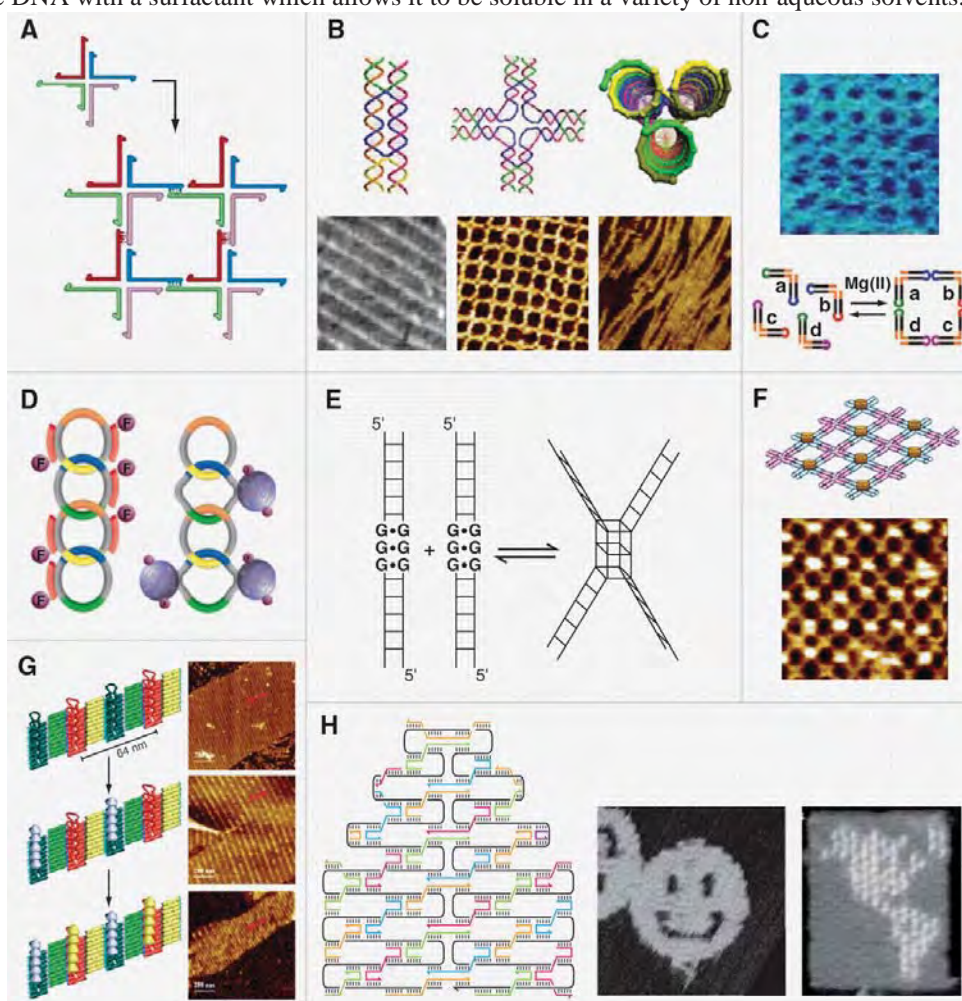

Figure 2: (A) Sticky-end motif for 2D structures. (B) Tile motif (left), four-way junction (middle) can result in a 2D square lattice; and helix bundles (right), can generate a 1D DNA nanotube. (C) Folded RNA molecules. (D) DNA circles form catenated ladder-assemblies. (E) Four-way junction composed of a guanine quadruplex. (F) A four-arm junction. (G) Four tiles are assembled into a 2D array. (H) DNA origami, this approach is used to access different 2D architectures. Image from F.A. Aldaye et al. Science 2008; 321: 1795-1799 reprinted with permission from AAAS.

1.2 DNA Surfactant Complexes

For these experiments a DNA–cationic surfactant complex is created using either hexadecyltrimethylammonium-chloride or bromide (CTMA). This surfactant complex renders the DNA water insoluble causing it to precipitate out of its aqueous solution and also has the added benefit of making the DNA more mechanically and thermally stable.[1] The DNA-CTMA complex is soluble in a variety of solvents including isopropanol, ethanol, methanol, acetonitrile, and butanol. Previous circular dichroism (CD) studies have shown that when using genomic DNA the DNA-CTMA compound retains its double helical structure in these solvents at temperatures over 100 ˚C.

1.3 Template Selection

The initial template, wagon wheel DNA (wwDNA), used for this research was chosen for its overall size.[10] Many different research laboratories have published sequences and protocols for creating a large variety of shapes and structures using differing numbers of DNA strands and different strand lengths.[10,11] Although, the potential template library is vast the initial template needed to have a void size of 10 nm or less and have a relatively large feature size (>15 nm) to facilitate visualization of the samples by atomic force microscopy (AFM). Furthermore, this template only consists of two DNA strands that combine to form a higher order structure using a T-junction motif which greatly simplifies the protocol for creating the initial template.

2. EXPERIMENTAL

2.1 Materials

RP-HPLC purified oligonucleotides were purchased from IDT DNA (Coralville, IA). DNA samples were reconstituted to a 100 μM final concentration using 1X IDTE (IDT DNA). All aqueous solutions were prepared with nanopure water (ddH$_2$O). All chemicals and supplies were purchased from Sigma-Aldrich or Invitrogen and were the highest grade and purity available. DNA sequences were chosen for their secondary structural characteristics as described in the literature.[10]

2.2 Methods

DNA Wagon Wheel Template Reconstitution: The specific DNA sequence was synthesized by IDT and packaged separately as single-stranded DNA, dubbed "Wheel 1" (sequence: 5′-TCC ACG GTC TGC TAC TCG GTA ATG GCT CAT CAA GCG TCC AGT TCC GCA AAC G -3′) and "Wheel 1 comp" (sequence: 5′- CGC TTC GTT TGC GGA ACT GGA GAT GAG CCA TTA CCG AGT AGG TGG ACA GAC C-3′).[10]
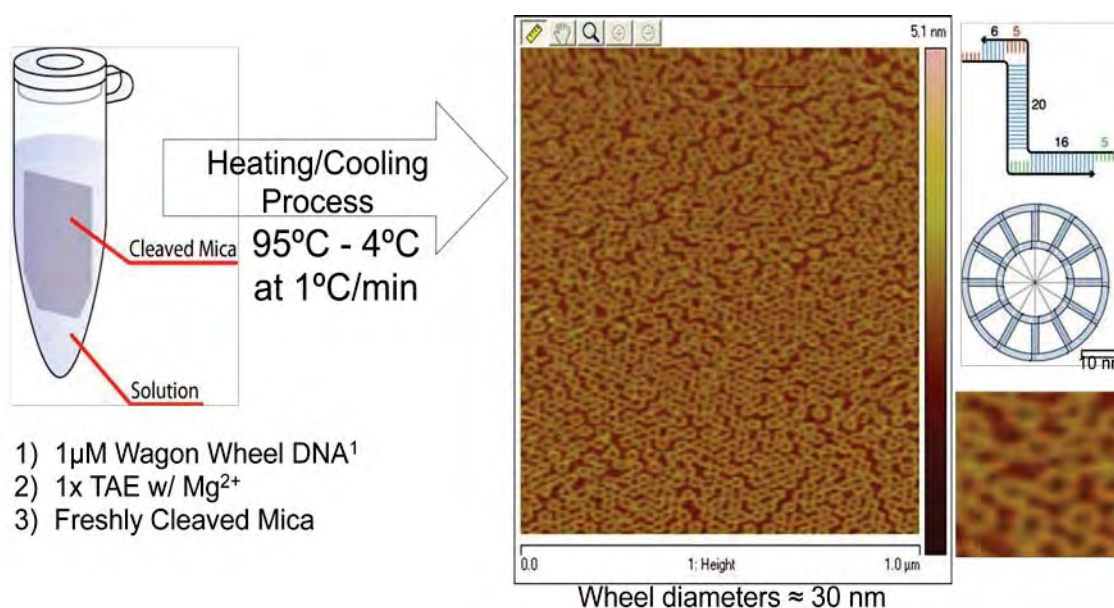

Figure 3. Schematic of the preparation of wwDNA as well as characterization via AFM.

The reconstitution of the two single strands of DNA into the double-stranded, tertiary wheel structure was accomplished with the use of a JASCO J-815 CD spectrometer (Easton, MD). 1X Tris-Acetate-EDTA (TAE) was diluted from 10X stock solution and combined with 12.5 mM Mg^{2+} (1 M stock), 2.5 µL Wheel 1 (100 µM stock in TAE), and 2.5 µL Wheel 1 Comp (100 µM stock in TAE), and samples (with and without freshly cleaved mica present) were incubated in a cuvette for CD studies and a microfuge tube for all other experiments. The wagon wheel complex once formed is referred to as wwDNA. As seen in Figure 3, the mica disk was placed in the microcentrifuge tube during its reconstitution process so as to provide even coating over the entire mica surface. The sample was heated to 95 °C and then cooled to 4 °C at a ramp rate of 0.1 °C per minute.

Creating the DNA-Surfactant Complex (DNA-CTMA): The process of the DNA-CTMA reaction has been previously studied and explained, and is only being reproduced in this experiment.[12-14] Both CTAB and CTAC, were reacted with the reconstituted DNA (salmon sperm and wagon wheel DNA) to form a complex that appeared as a white, solid pellet that was found to be insoluble in water. The samples were then centrifuged for 5 minutes at a speed of 7500 rpm and at a temperature of 4 °C. The resulting supernatant was placed in a collection tube to determine efficiency of complex formation and the pellet was rinsed with ddH_2O. The centrifugation, supernatant collection, and rinsing steps were repeated two additional times. The samples were placed in an Eppendorf Vacufuge (Hauppauge, NY) and lyophilized for about one hour until samples were dry. The CTAC was chosen over CTAB for future experiments because it is completely soluble at room temperature whereas the CTAB precipitates at room temperature in aqueous solution and requires an additional heating step.

Circular Dicroism (CD) Spectroscopy: The formation of the wagon wheel template in both aqueous and non-aqueous solutions was monitored via CD spectroscopy using a Jasco 850 CD spectrometer (Easton, MD) using the temperature interval program. The temperature interval program takes a CD and UV scan at preset temperature intervals (as determined by the user) allowing for direct monitoring of the sample at different temperatures. In addition to direct monitoring of the DNA and DNA-CTMA complex formation, the supernatant of each sample in the DNA-CTMA preparation was analyzed using both CD and UV spectrometry in order to determine the efficiency of the DNA-CTMA complex formation. The absorbance for each sample were tracked (see Table 1) to determine the remaining concentrations of DNA left in the supernatant after rinsing the pellet with water.

Table 1: Effect of surfactant concentration on the maximum relative absorbance

Solvent Used in CTAB solution	Final Concentration of CTAB (w/v)	Amount of DNA (µL)	Max. Relative Absorbance
0.5 M NaCl	0.10%	85	.578
	0.50%	85	.301
	1.00%	85	.015
	1.50%	85	-1.159
ddH_2O	0.10%	85	.925
	0.50%	85	.799
	1.00%	85	.679
	1.50%	85	.509

Testing Solubility in Organic Solvents: Lyophilized sample pellets were resuspended in different organic solvents: isopropyl alcohol, ethanol, methanol, acetonitrile, and butanol (see Table 2).

Sample Mounting: The samples were mounted on silicon wafers or incubated with freshly cleaved mica in solution. The DNA and DNA-CTMA samples on the silicon wafers were either spin-cast or drop-cast onto the silicon surface. To spin-cast the sample, about 150 µL of the DNA sample was placed onto a silicon wafer and spun in a Laurell Technologies Corporation Spin Processor (North Wales, PA) at speeds ranging from 1000 - 8000 rpm. For drop-casting, 20-50 µL of the DNA sample was added drop-wise by micropipette onto a silicon wafer and the liquid was allowed to

either evaporate under room temperature conditions or samples were incubated for 30 minutes followed by a rinse with ddH$_2$O and dried under N$_2$. Sample uniformity and surface roughness was then characterized by AFM and SEM.

Table 2. Solubility of surfactant complexes in different organic solvents

CTAB vs. CTAC	Organic Solvent	Volume (μL)	Soluble at RT
CTAB in 0.5M NaCl	Isopropyl Alcohol	250	NO
CTAB in 0.5M NaCl	Acetonitrile	200	NO
CTAB in 0.5M NaCl	Ethanol	200	NO
CTAB in ddH$_2$O	Isopropyl Alcohol	250	YES
CTAB in ddH$_2$O	Acetonitrile	200	NO
CTAB in ddH$_2$O	Ethanol	200	YES
CTAC in ddH$_2$O	1-Butanol, anhydrous	200	YES
CTAC in ddH$_2$O	Isopropanol, anhydrous	200	YES
CTAC in ddH$_2$O	Ethanol, anhydrous	200	YES
CTAC in ddH$_2$O	Methanol, anhydrous	200	YES
CTAC in ddH$_2$O	Acetonitrile	200	YES

Surface Characterization of DNA-Surfactant Complex by Atomic Force Microscopy (AFM): Veeco Nanoman V SPM Atomic Force Microscope (Santa Barbara, CA), was used in tapping mode with Bruker OTESPA tips (Santa Barbara, CA) to image the reconstituted DNA samples.

Surface Characterization of DNA-Surfactant Complex by Ellipsometry: J.A. Woollam M-2000 Spectroscopic Ellipsometer (Lincoln NE) was used to measuring the thickness of the DNA thin films on SiO$_2$ following standard procedures as provided by the instrument application engineers at Woollam.

3. RESULTS AND DISCUSSION

3.1 Wagon Wheel Template Aqueous

The DNA wagon wheel (wwDNA) construct was successfully made in aqueous solution. The characterization of the aqueous wwDNA samples was conducted by AFM and CD spectroscopy. The wwDNA samples were co-incubated in aqueous solution with freshly cleaved mica and then imaged via AFM. As illustrated in Figure 4, the size of the features are in good comparison with literature reports and are approximately 30 nm in diameter, with a 10 nm void, and a 2.5 nm feature height.[10] In addition, upon further AFM scanning, it appears that > 90% of the sample is covered in a similar packing density to that illustrated in Figure 4 which are also consistent with the reported literature of template assisted DNA self assembly on mica.[10-11] This promising result led to further experiments to allow for monitoring of the wwDNA assembly process in solution.
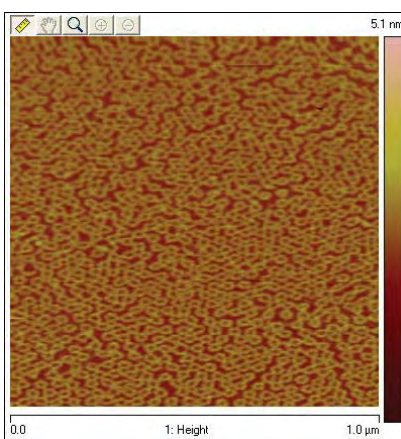

Figure 4: wwDNA created using template assisted sample annealed in solution (TAE-Mg2+)

We determined that AFM and CD spectroscopy data could be coupled and used as complementary techniques in order to measure the assembly in solution. Figure 5 illustrates that we can monitor the assembly of higher order wwDNA structures via circular dichroism. As the temperature of the sample decreases slowly over time, the secondary structural features of the DNA become more prevalent. These positive results with the aqueous wwDNA allowed for optimization and characterization of wwDNA-CTMA complex under non-aqueous conditions. The successful reconstitution of the wwDNA, the next step was to attempt to solubilize the wwDNA in non-aqeuous solutions.
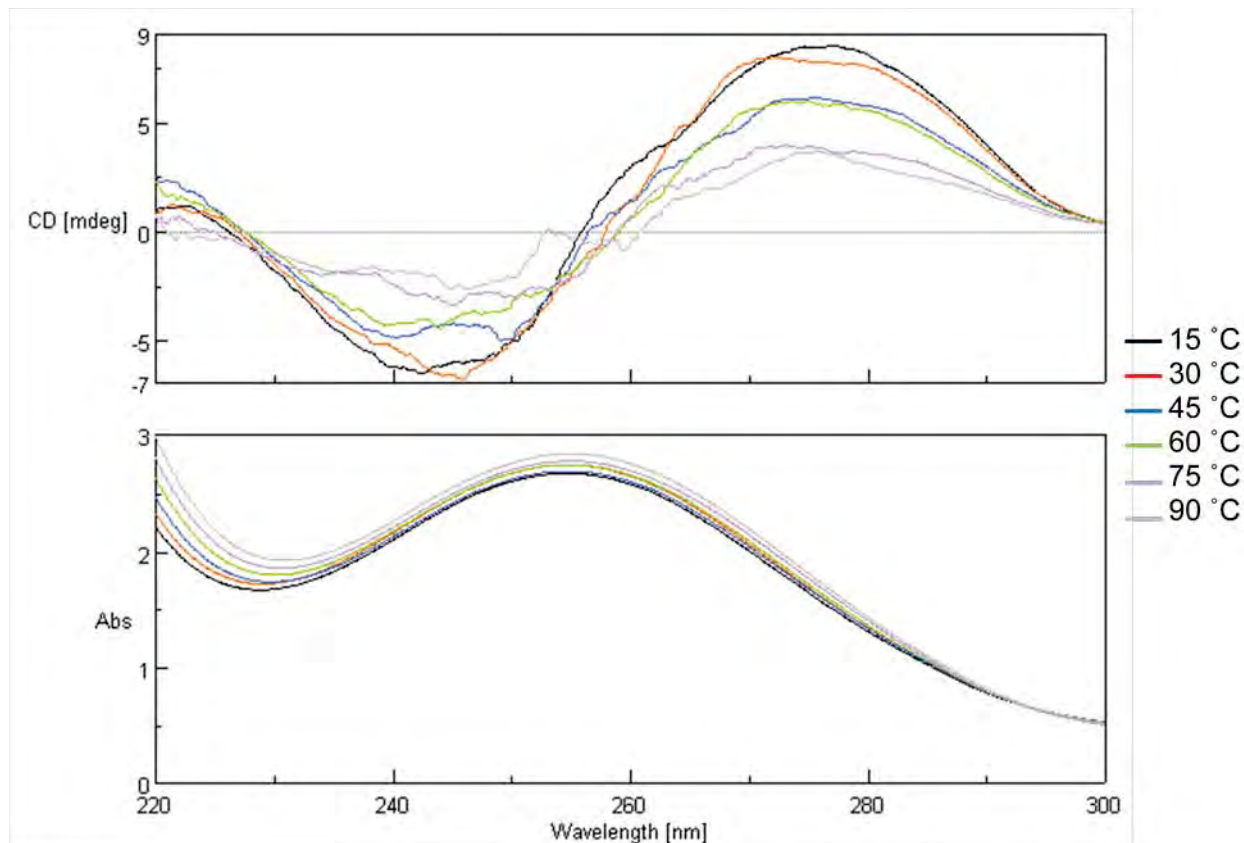

Figure 5: CD and UV spectroscopy of wwDNA in aqueous solution.

3.2 DNA-CTMA Control Experiments and Solvent Selection

In order to validate our experimental techniques under non-aqueous conditions the preliminary experiments were conducted on readily available genomic salmon sperm DNA (saDNA). These initial experiments allowed for an inexpensive, conclusive way to test methods and procedures and directly compare results with the published literature.[14] The saDNA samples were reacted with two different surfactant solutions at different concentrations, in order to determine the optimum conditions for future experiments. These saDNA experiments illustrated that the CTAC was more appropriate for future experiments due to its water solubility at room temperature. Additionally, 1-Butanol was chosen as the solvent for future experiments due to its high boiling point and lack of absorbance in the active CD spectroscopy region. Once the DNA-surfactant complex formed, the saDNA-CTMA samples were characterized via CD spectroscopy and AFM with 1-butanol as the solvent. Figure 6 illustrates that the structure of the saDNA does not change upon creation of the saDNA-CTMA complex. The saDNA-CTMA was then both drop cast and spin cast onto both freshly cleaved mica and silicon wafers for characterization via AFM. As illustrated in Figure 7, although neither sample shows any discernable features, the spin cast sample is appreciably smoother and more uniform than the drop-cast sample. These results and experimental controls conducted with the saDNA paved the way for experiments with the wwDNA.
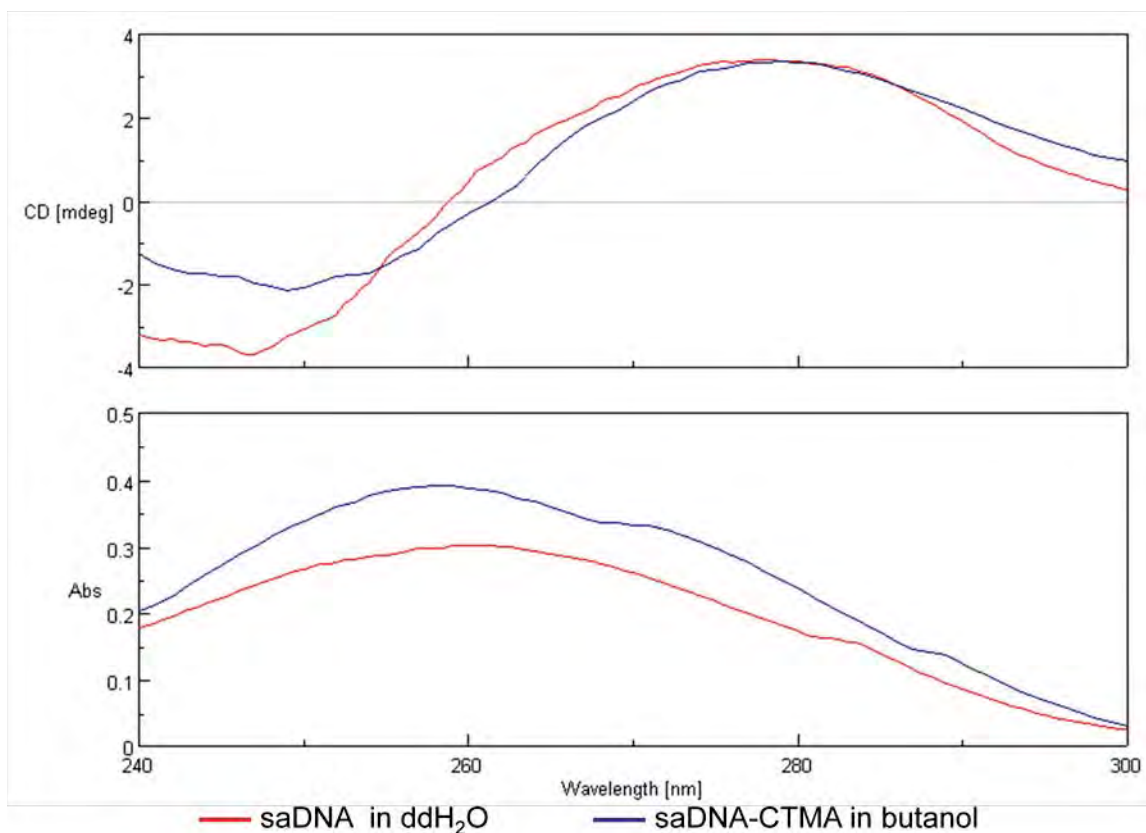
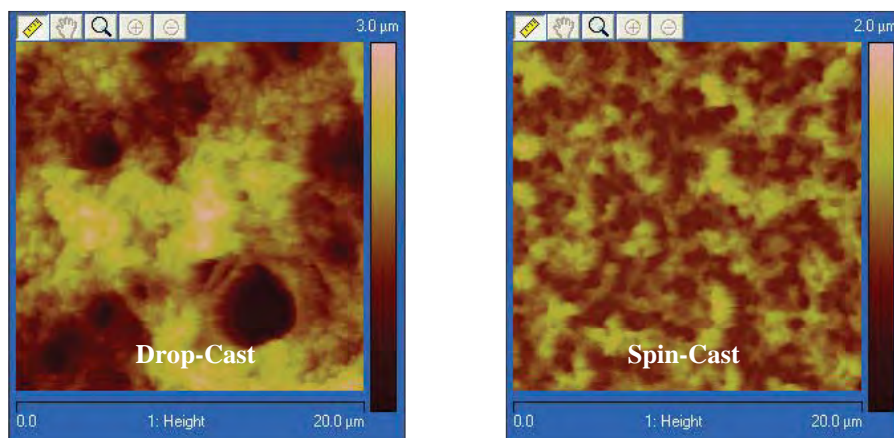Figure 6: CD spectroscopy of SaDNA (red) in aqueous solution and saDNA-CTMA (blue) in butanol.

Figure 7: AFM Picture: Spin-Cast vs. Drop-Cast saDNA Samples on freshly cleaved mica.

3.3   wwDNA-CTMA Experiments

The experimental procedures outlined for the saDNA-CTMA characterization were repeated with the wwDNA-CTMA. As illustrated in Figure 8, the CD spectrum suggests that the double helical structure of DNA is preserved in the wwDNA-CTMA complex. Although the spectrum from aqueous to butanol is slightly shifted, this shift is in agreement with literature reports the overall shape of the spectra remains the same.[15]   The wwDNA-CTMA samples were then both drop cast and spin cast onto freshly cleaved mica. (Figure 9) While these processing of these samples needs to be further optimized the samples do appear to have smaller, more organized structural features which is consistent with the wwDNA structure.
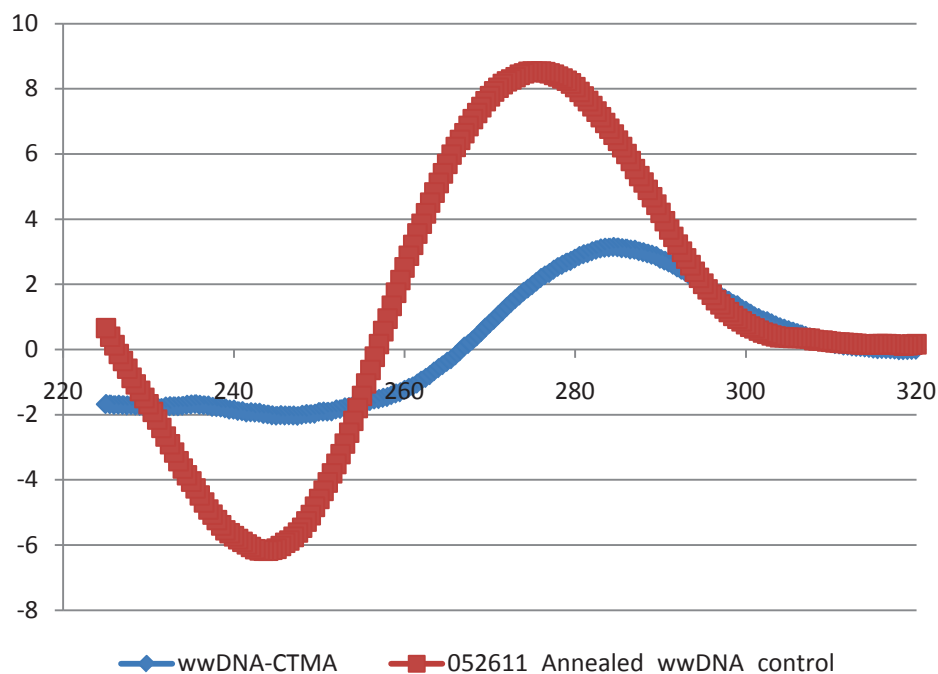
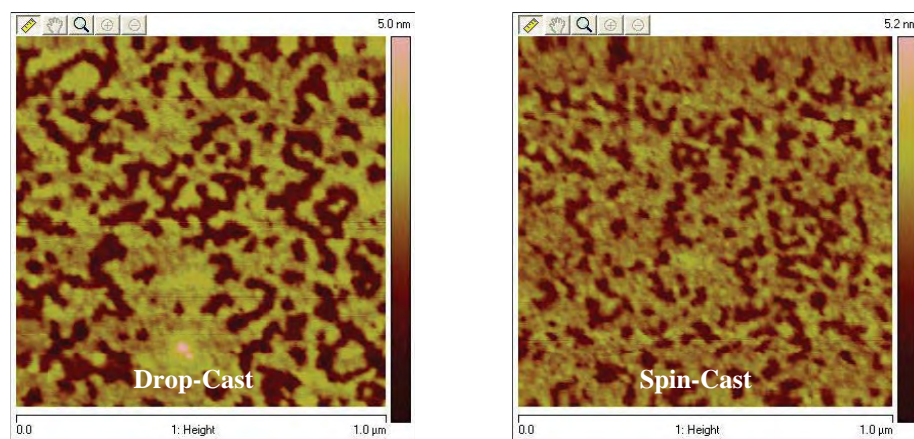Figure 8: CD spectroscopy of wwDNA assembled in aqueous solution (red) vs. wwDNA-CTMA complex in butanol.

Figure 9: Wheel DNA-CTMA complex (10 µL, dissolved in 1-butanol) drop-cast on freshly cleaved mica.

4.   CONCLUSION

Template assisted wwDNA structures were formed in aqueous solution and characterized via AFM and CD spectroscopy. The wwDNA samples were then combined with surfactant CTAC to form a DNA-CTMA complex. This wwDNA-CTMA is soluble in a variety of organic solvents and was characterized via AFM and CD spectroscopy. This novel material has great promise for future materials templating applications as illustrated in figure 10.

Future work will include optimization of the material surface for single layer deposition of the templated DNA-CTMA complex as well as transitioning to a wide range of structures. These materials have great promise in creating bottom-up features smaller than current top-down lithographic techniques allow.
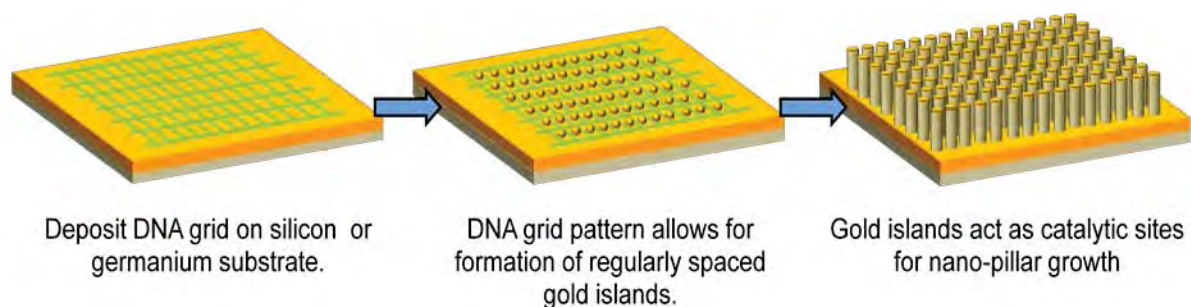
Figure 10: Future research strategy for surface assembled DNA structures.

ACKNOWLEDGEMENTS

REFERENCES

[1] Singh,T. B., Sariciftci, N. S. and Grote, J. G., "Bio-organic optoelectronic devices using DNA," Adv. Polym. Sci. 223, 189-212 (2010).

[2] Heckman, E., "Biotronics for defense," SPIE Professional April, 20-22 (2011).

[3] Seeman, N. C., "Nanomaterials based on DNA," Annu. Rev. Biochem. 79, 65-87 (2010).

[4] Han, D., Pal, S., Nangreave, J., Deng, Z., Liu, Y. and Yan, H., "DNA origami with complex curvatures in three-dimensional space," Science 332, 342-346 (2011).

[5] Lin, C., Liu, Y. and Yan, H., "Designer DNA nanoarchitectures," Biochemistry 48(8),1663–1674 (2009).

[6] Endo, M. and Sugiyama, H. "Chemical approaches to DNA nanotechnology," Chembiochem 10(15), 2420-2443 (2009).

[7] Douglas, S. M., Dietz, H., Liedl, T., Hogberg, B., Graf, F. and Shih, W. M. "Self-assembly of DNA into nanoscale three-dimensional shapes," Nature 459, 414-418 (2009).

[8] Tan, S. J., Campolongo, M.J., Luo, D. and Cheng, W., "Building plasmonic nanostructures with DNA" Nat. Nanotechnol. 6, 268-276 (2011).

[9] Service, R.F., "DNA nanotechnology grows up," Science 332(6034), 1140-1143 (2011).

[10] Hamada, S. and Murata, S., "Substrate-assisted assembly of interconnected single-duplex DNA nanostructures," Angew. Chem. Int. Ed. 48, 6820-6823 (2009).

[11] Sun, X., Ko, H. S., Zhang, C., Ribbe, A. E. and Mao, C., "Surface-mediated DNA self-assembly," J. Am. Chem. Soc. 131(37), 13248-13249 (2009).

[12] Hagen, J. A., Grote, J. G., Ogata, N., Zetts, J. S., Nelson, R. L., Diggs, D. E., Hopkins, F. K., Yaney P. P., Dalton, L. R. and Clarson, S. J., "DNA photonics" Proc. SPIE 5351, 77-86 (2004).

[13] Tanaka, K. And Okahata, Y., "A DNA-lipid complex in organic media and formation of an aligned cast film," J. Am. Chem. Soc. 118(44), 10679-10683 (1996).

[14] Hagen, J. A., Grote, J. G., Singh, K. M., Naik, R. R., Singh, T. B. and Sariciftci, N. S., "Deoxyribonucleic acid biotronics," Proc. SPIE 6470, 64700B (2007).

[15] Grote, J. G., Air Force Research Laboratory – Materials and Manufacturing Directorate, Tri-Service Biotechnology Technical Planning Meeting, February 23-24, 2010.

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